AMERICAN JOURNAL OF PHARMACY VOL. 130 NOVEMBER 1958 NO. 11

AMERICAN Journal of Pharmacy

AND THE SCIENCES SUPPORTING PUBLIC HEALTH

Dr. Alfonso R. Gennaro and Dr. Arthur Osol
Examining Spectrogram Obtained With
Beckman IR-4 Infrared Spectrophotometer
(See article p. 368)

Since 1825 *November 1958*

American Journal of Pharmacy

Published monthly by the Philadelphia College of Pharmacy and Science
43d Street, Kingsessing and Woodland Avenues, Philadelphia 4, Pa.

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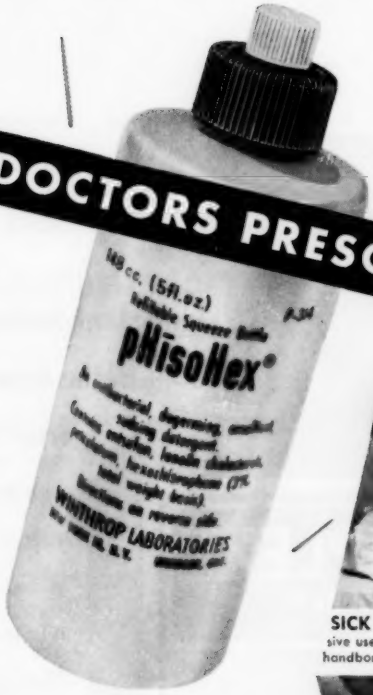

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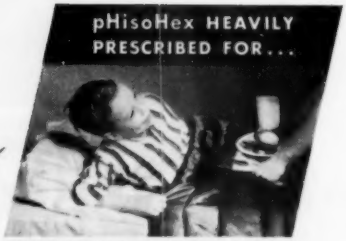

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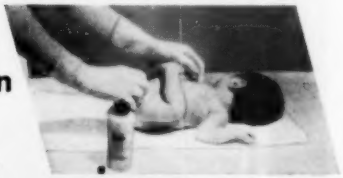

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Vol. 130 NOVEMBER 1958 No. 11

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E D I T O R I A L

A SOUND PROPOSAL

ACCORDING to all reports, not too much was accomplished at the all-day meeting held in Chicago just prior to the recent Interim Meeting of the House of Delegates of the American Pharmaceutical Association. This meeting was held pursuant to a resolution adopted at the last Annual Meeting of the House of Delegates and it was for the purpose of discussing what efforts should be directed toward the protection of public health by limiting the sale of potentially harmful drugs to pharmacists. At this meeting, many of Pharmacy's spokesmen voiced their strong disapproval of the current trend whereby more and more products which have long been the almost exclusive province of pharmacy are now finding their way into all kinds of retail outlets. Only one really concrete proposal was made, however, which would seem to be a positive step along lines which might accomplish something rather than simply continue Pharmacy's position of offering vociferous criticism to the present trend. This suggestion was made by the Secretary of the American College of Apothecaries. This organization, while it is relatively small, has been a very influential and constructive agency for the betterment of pharmacy and improvement of its professional status. The Secretary, Mr. Robert E. Abrams, made the following recommendations: First, that "an all-out effort be made to adopt an amendment to the Federal Food, Drug and Cosmetic Act to limit the sale of all drug products for internal administration and all external products capable of masking disease or delaying treatment to pharmacists;—and the establishment of an F. D. A. Advisory Committee representing Pharmacy, Medicine, Public Health and other interested groups to establish which products might be safely sold by non-pharmacists." If this is not found feasible, it was then suggested that organized pharmacy "sponsor an amendment to the Food, Drug and Cosmetic Act which would limit the distribution to pharmacists of any drug product that at one time bore the prescription legend or that contains drugs which have at one time been on the legend basis. Thus, these so-called 'delegendized' products would be sold only under the professional supervision of a pharmacist."

The need for such action is long overdue now that we have had the opportunity to appreciate fully the impact which the Durham-

Humphrey Amendment has had on the distribution of drugs and medicines in the United States. This Amendment makes possible only two kinds of drug classification—those dispensed only on prescription and those sold over the counter. In the case of the latter classification, federal law makes no distinction whatsoever as to what kind of a retail outlet through which these drugs may be sold. While there may be some persons in the Food and Drug Administration who feel that many OTC drugs should be sold only by pharmacists, there is presently no legislative basis for insisting that this be done. A few state laws attempt to prevent the promiscuous sale of drugs but many manufacturers with more concern for profit than the public welfare have used the failure of the federal government to restrict such sale as an argument that there is nothing wrong with the promiscuous sale of such drugs. This is patently untrue but wherever pharmacists have attempted to prevent it they have been accused of being motivated solely by self-interest rather than public welfare.

The time has surely come when, through some legislative approach, the profession of pharmacy should attempt to place this matter in proper perspective and enlist the support of other groups who have a stake in, and a professional interest in, public health and welfare. No-one who gives this matter honest, unbiased, and well-informed attention can come to the conclusion that no professional training is necessary for the proper sale and distribution of drug products. There are very rigid controls on the distribution of products and services far less dangerous than drugs yet some potentially dangerous products are being sold today with no professional supervision whatsoever.

We not only endorse the proposal made by Secretary Abrams but we suggest that the other professional organizations of pharmacy take appropriate action in giving this proposal their support. The time has come when pharmacists must assert their professional instincts and enlist the support of others so motivated. For too long, some of our "leaders" in pharmacy have advocated a careful course of expediency and compromise rather than to risk offending some of the economic interests from which they derive their support. The time, we fear, is long overdue when those who wish to see our profession go forward should stand and be counted, as well as counted upon to back efforts such as this which must be made.

L. F. Tice

INFRARED SPECTROPHOTOMETRY AS A TESTING PROCEDURE OF U. S. P. XVI

By Alfonso R. Gennaro, Ph.D.,* and Arthur Osol, Ph.D.**

THE infrared region of the electromagnetic spectrum, the region characterized by the thermal effect of its radiation, has provided in recent years an extraordinarily fertile field for research, one in which the advances have been commensurate with the more spectacular and highly specialized discoveries and inventions in other areas. Infrared technology is utilized in night photography, in missile guidance systems, in measuring the temperatures of steel furnaces, and in many other important applications.

For the chemist, infrared has made possible the development of one of his most useful instrumental procedures—infrared spectrophotometry. With it, the determination of the molecular structure of a pure substance is facilitated, and qualitative and quantitative analyses may usually be performed with ease. In the U. S. P. XVI, infrared spectrophotometry will be used as an identification procedure for numerous official substances.

It is the purpose of this article to explain the basic principles of infrared spectrophotometry, the design and operation of a typical instrument, the underlying principles of infrared spectrophotometric measurement techniques and, finally, to provide illustrations of types of infrared identification procedures to be utilized in the forthcoming revision of the *United States Pharmacopeia* (U. S. P. XVI).

Basic Principles of Infrared Spectrophotometry

Molecules, regardless of the physical state or nature of the substance they comprise, are in a dynamic state. Besides the translational motion of the molecule, the electrons within the molecule vibrate around and between the two or more positively charged atomic nuclei of the molecule, and the nuclei themselves move not only as a unit but vibrate with respect to each other, and also rotate about the center of

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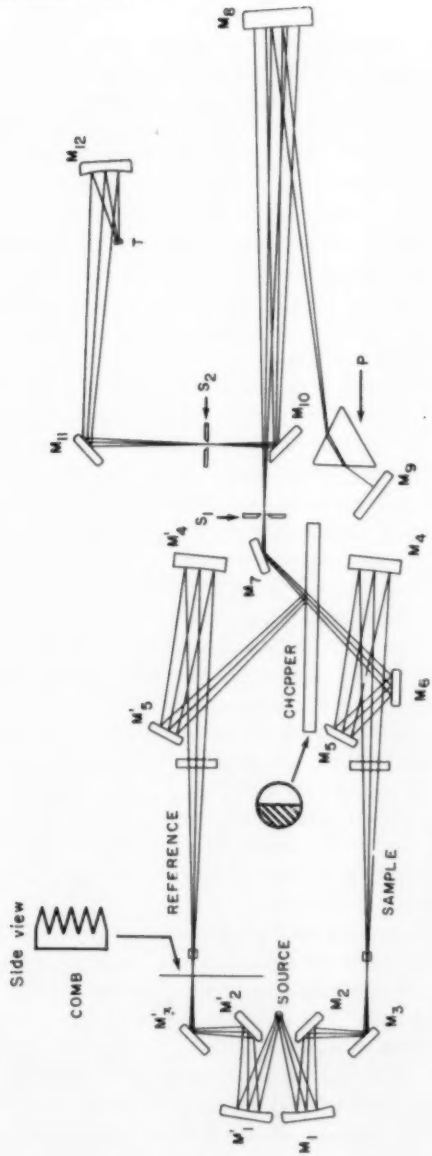


Courtesy Beckmon Process Instruments Div.

FIGURE 1.

SCHEMATIC DIAGRAM OF OPTICAL SYSTEM OF A DOUBLE BEAM INFRARED SPECTROPHOTOMETER.

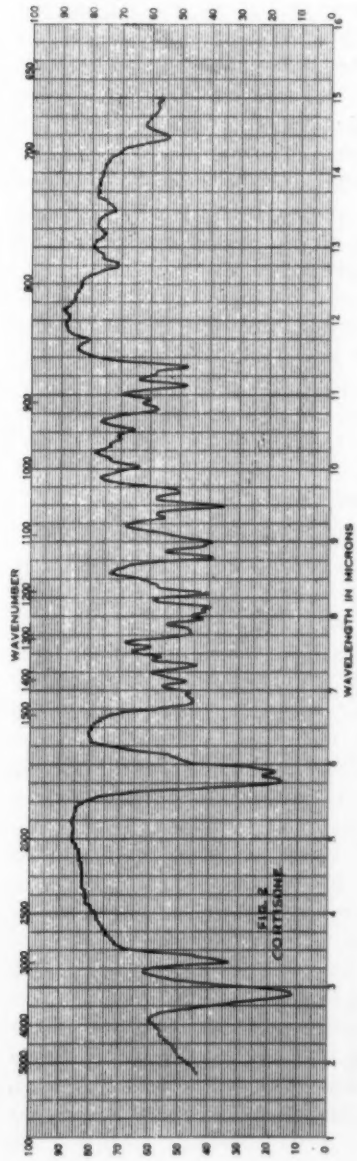
gravity of the molecule. All these motions occur with fixed and characteristic frequencies, and under proper experimental conditions give rise to absorption spectra characteristic of the compound.

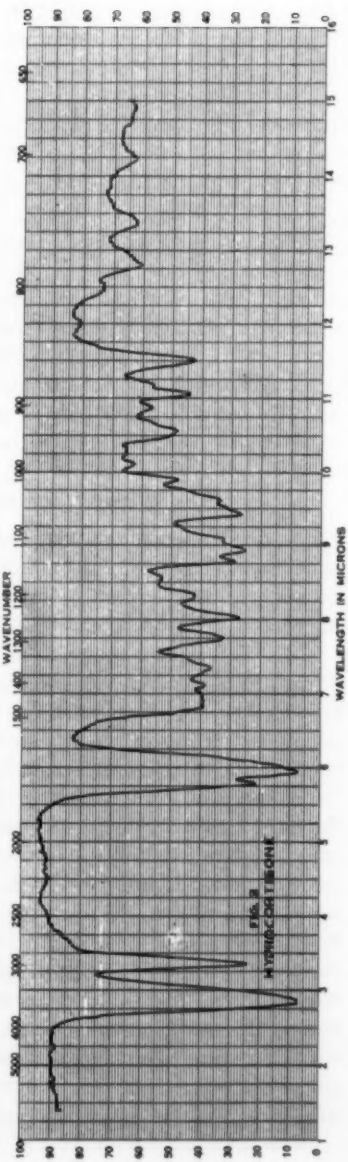
The energy required to produce *rotation* of molecules is of low magnitude, corresponding to that of long or far infrared radiation (wave length about 200 microns). To effect *vibration* of atoms or nuclei in molecules, however, the greater energy available in the shorter or near infrared region (about 0.5 to 25 microns) is required; of course, this energy is more than enough to cause, simultaneously, rotation of molecules as well. The energy required to produce vibrations of electrons is still greater, and corresponds to the energy available in visible and ultraviolet light.

The behavior of a substance in the near infrared region, as defined above, is especially productive of valuable information about the substance. If a beam of infrared energy of continuously changing wave length is passed through a substance, the intensity of the transmitted energy will not be constant but will vary with the wave length, indicating preferential absorption by the substance of certain portions of energy in a manner characteristic of the molecular composition and configuration of the substance; this absorption pattern is reproducible under constant experimental conditions. If the intensity of the transmitted energy is plotted against the wave length of the incident energy while the substance is being continuously "scanned", an infrared absorption spectrum results (Figures 2 to 7).

The absorption maxima (also called absorption bands), which correspond to transmittance minima, are more or less characteristic of specific groups or pairs of atoms in a molecule. These maxima are evidence of the ability of certain arrangements of particular atoms in the molecule to absorb energy at characteristic wave lengths, the degree of absorption depending on the binding forces between the atoms and also on the masses of the atoms.

The position of the absorption band for a particular pair or group of atoms is not entirely constant for the same pair or group when it occurs in different molecules, deviations arising because of the influence of neighboring atoms. For example, the absorption bands arising from C-H stretching vibrations occur at 3.3 to 3.7 microns in aliphatic compounds but at 3.2 to 3.3 microns in aromatic compounds. The position of the carbonyl (C=O) absorption band is influenced by the type of structure in which it is found, falling at


WAVENUMBER
WAVELENGTH IN MICRONS
FIG. 2
CORTISONE

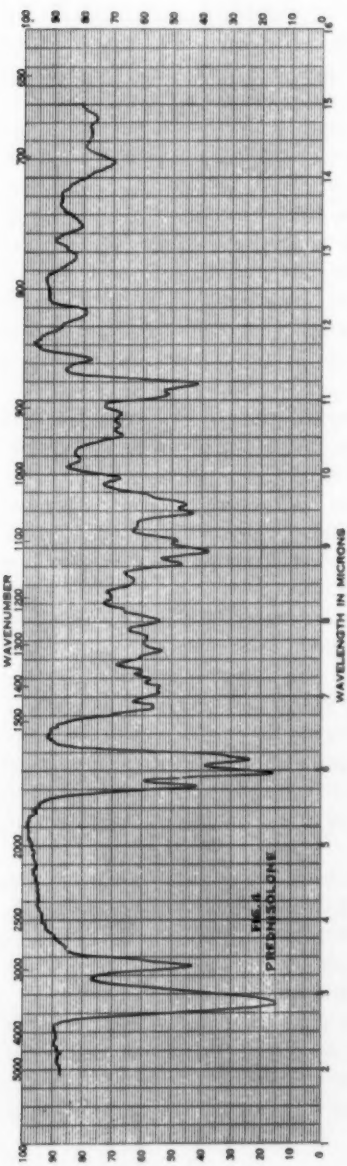

WAVENUMBER
WAVELENGTH IN MICRONS
FIG. 3
HYDROCORTISONE

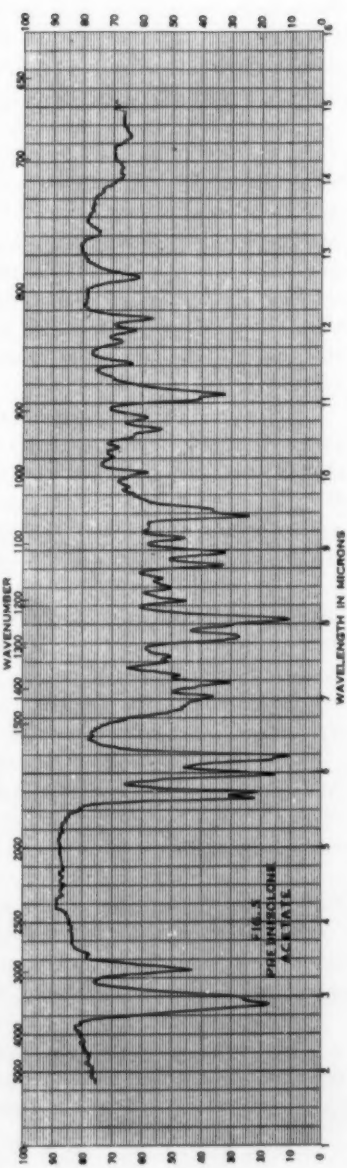
5.75 to 5.80 microns for aldehydes and ketones, at 6.0 to 6.1 microns for acids, at 5.75 to 5.85 microns for esters, and giving rise to two bands for anhydrides (since two carbonyl groups are present), at 4.9 to 5.05 microns and 5.60 to 5.75 microns, respectively. Regardless of this seemingly inexact location of the absorption bands for various atomic pairs or groups, the experienced spectroscopist can ascertain important items of structural information about a compound under investigation. First, the presence, or absence, of any particular atomic grouping can be determined; second, almost positive identification of a molecule may be made if the spectrum of the unknown can be matched with that of a known compound or compared with the spectra of a series of closely related "model" compounds. Yet, it is possible for two or more compounds that differ only slightly in structure, *e.g.*, homologous aliphatic hydrocarbons, to have *almost* identical spectra. In such cases, the availability of other physical and chemical data for the unknown facilitates its positive identification.

Instrumentation Principles

Several infrared spectrophotometers which automatically plot per cent transmittance (or optical density) versus wave length (or frequency) are currently available commercially. A schematic diagram of a typical "double beam" instrument is shown in Figure 1.

The infrared radiation from a suitable source (a Globar unit, Nernst glower, or Nichrome coil) is divided into two beams by mirrors M_1, M_2, M_3, and M_1', M_2', M_3', then passed through the sample and reference compartments, respectively. The beam emerging from the sample compartment is reflected by mirrors M_4, M_5, M_6 through the unsilvered portion of a rotating half mirror, called a chopper, to mirror M_7. The beam emerging from the reference compartment is reflected by mirrors M_4', M_5' to the silvered portion of the chopper, and thence to mirror M_7. The function of the chopper is to send energy alternately from the sample and reference compartments through the rest of the optical system, collectively known as the monochromator, to the thermocouple or bolometer for measurement. The sequence is as follows: after passing through the entrance slit S_1, the radiant energy is directed by spherical collimator M_8 through the Littrow prism assembly, consisting of P and M_9, which is slowly rotated to cover the desired spectral range (usually 2 to 15 microns). Mirror M_9 reflects the energy back through prism P, and M_8 reimages


WAVENUMBER
WAVELENGTH IN MICRONS
FIG. 4
PREDNISOLONE


WAVENUMBER
WAVELENGTH IN MICRONS
FIG. 5
PREDNISOLONE ACETATE

the dispersed radiation, with the aid of mirror M_{10}, through exit slit S_2, thence to M_{11} and M_{12} to form an image on the detector T (a thermocouple or bolometer). It is apparent that T receives energy impulses alternately from the sample and reference compartments.

As it is not possible to obtain a constant amount of radiant energy from the source, the two slits S_1 and S_2 are automatically widened or narrowed in order to achieve a relatively constant level of energy over the desired spectral range. Another function of the slits is to limit the band of energies traversing the monochromator, whereby the spectral purity of the radiation striking the detector is increased. The rotating Littrow prism assembly is mechanically or electronically coupled to a paper strip chart moving in synchronism with it, the abscissa of the chart being calibrated in wave length or frequency units.

The intensity of radiation received from the reference cell is generally greater than that from the sample cell, because the sample absorbs a portion of the incident energy. To compensate for this, the detector is electronically linked to an optical attenuator or comb (so named from its resemblance to a comb), which is mechanically inserted into the reference beam to reduce its intensity. Fluctuations in the radiation transmitted by the sample over the range of wave length covered by the Littrow prism assembly are compensated through the proper degree of attenuation of the reference beam by the comb. A pen is connected to the comb, either by electronic or mechanical means, the pen following the lateral motion of the comb at different wave lengths and tracing this motion on the axis of ordinates of the paper strip chart either as per cent transmittance or optical density at the wave length indicated on the axis of abscissas. The tracing constitutes the infrared absorption spectrum of the substance in the sample cell.

A double beam instrument, such as the one described, has the important advantage, over a single beam instrument, that atmospheric absorption of energy (due to water vapor, carbon dioxide, etc.) is automatically cancelled and does not appear superimposed on the spectrum of the substance being examined.

It is imperative that containers used for the sample and in the reference beam, as well as the prism, should be transparent to infrared radiation so as to provide maximum transfer of energy through the spectrophotometer. For this reason, it is impossible to use glass as

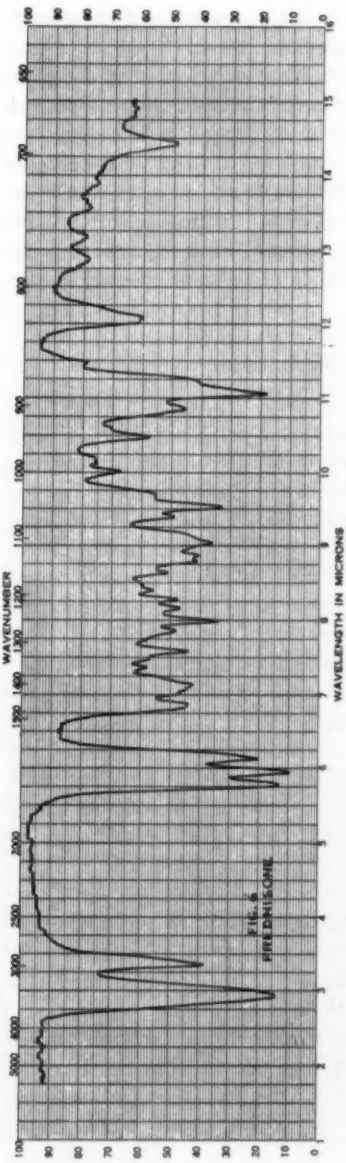


FIG. 6
PREDNISONE

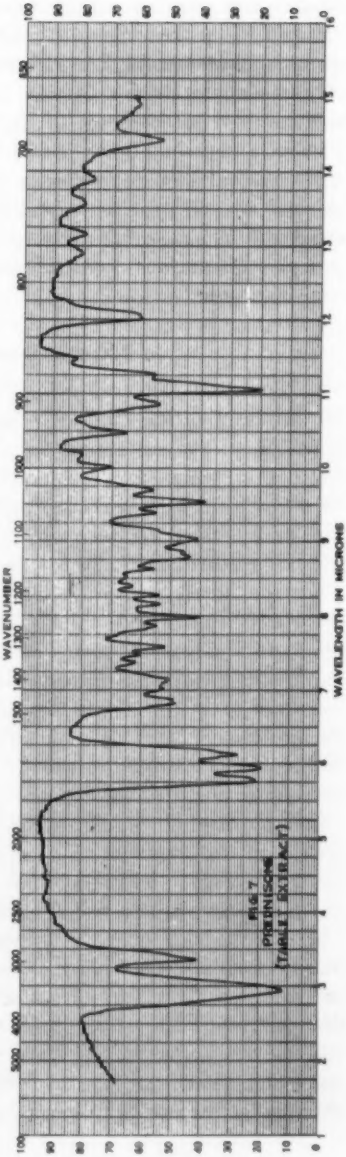


FIG. 7
PREDNISONE
(7% CHLOROFORM EXTRACT)

the material for constructing containers and prisms. Sodium chloride (rock salt) is the substance most commonly used for this purpose, being employed in the 2- to 15-micron wave length range; yet, it has certain disadvantages. Quartz, calcium fluoride, or lithium fluoride may be used in the range of shorter wave lengths, while potassium bromide and cesium bromide may be employed at longer wave lengths. Only quartz, calcium fluoride, and lithium fluoride are relatively unaffected by moisture, but because of their limited useful spectral range and high cost they are usually used only in special studies. Use of aqueous solutions in containers made of sodium chloride is obviously precluded.

Measurement Techniques

Gases and pure liquids are the simplest to handle in preparation for determining their infrared absorption spectra, for all that is required is to place a sample in a cell of suitable dimensions, having parallel end windows of sodium chloride or other infrared-transparent substance, and then determine the spectrum. The effect of concentration on a gaseous sample may be investigated by diluting the sample with an unreactive, non-absorbing gas. The technique is not so simple when the liquid, or solid, sample is dissolved in a solvent, for the latter may itself absorb infrared energy. In the double beam spectrophotometer, advantage may sometimes be taken of the fact that absorption by the solvent may be *essentially* compensated for by placing a cell containing solvent only in the reference beam. It is apparent that the problem of selecting a suitable solvent medium involves not only finding a liquid having solvent action but also having sufficient transparency in the infrared. If the solvent absorbs considerable energy (has too many intense absorption bands), opaque or "dead" segments appear in the spectrum as a consequence of complete absorption of incident energy. This condition cannot be compensated for by the use of solvent in the reference beam of a double-beam instrument. The disadvantage may be circumvented, however, by using two or more solvents, each having a "window" in the infrared region, to prepare separate, single-solvent solutions of the sample. A portion of the spectrum is determined in the transparent segment of each solvent, and the respective portions are put together to form the complete spectrum. For example, by virtue of the fact that carbon tetrachloride is essentially transparent up to 7 microns, while carbon

disulfide is transparent in the region of 7 to 15 microns, it is possible to obtain a complete infrared absorption spectrum of a sample, free from interference by solvent, by combining the separate spectra run in each solvent. In many cases, only a small part of a spectrum is of interest; then a suitable solvent having a "window" in the region of interest is selected.

Often, it is impossible to find a solvent possessing the characteristics mentioned above, for which reason a variety of other methods for determining infrared spectra have had to be devised. A method very commonly employed is that referred to as the "mull" technique, in which the sample is ground (mulled) with a weakly absorbing, nonvolatile liquid (generally mineral oil) in which the sample is relatively insoluble, and the resulting dispersion is mounted between two plates of rock salt for measurement of the spectrum. Mineral oil has the disadvantage of absorbing at 3.4, 6.8, and 7.2 microns, which are the C-H absorption wave lengths. If it is desired to study the segments of a spectrum in these particular areas, a completely fluorinated hydrocarbon, which obviously contains no C-H bonds, may be used as the mulling agent. Quantitative evaluations are difficult to achieve with mulls because of the impracticality of (1) completely removing the mulled paste from the mixing mortar or of obtaining an aliquot of it, and (2) measuring the exact thickness of the mulled sample.

In recent years the "pressed pellet" or "pressed disc" technique has been widely investigated. Essentially, this method consists in grinding the sample with finely powdered, dry potassium bromide (which is infrared-transparent) and then compressing the mixture under high mechanical pressure, in a vacuum, into a special die to produce a transparent disc which can be made of known concentration and the thickness of which can be measured readily with a micrometer; the infrared absorption of this disc arises solely from the sample component. A serious drawback of this seemingly ideal technique arises when a compound is polymorphic, this causing appearance of extraneous absorption bands resulting from alteration of the crystal structure of such compounds. Such difficulties may arise whenever a solid sample, *per se*, is used, as any prism spectrometer is in effect a partial polarizer. Polymorphism and crystal orientation effects may cause the appearance, disappearance and/or shifting of absorption bands. Many other methods of preparing solid samples, including formation of powder films, films from solutions, and melted or

mechanically prepared films, are used in special studies or when other methods of sample preparation are found to be unsatisfactory.

Infrared Spectrophotometry in U.S.P. XVI

In the preceding, it is stated that almost positive identification of a substance may be made from its infrared absorption spectrum, which may be regarded as a "fingerprint" of the substance. Moreover, the intensity of absorption of a substance at a suitable wave length may be used for a quantitative measurement of it.

The forthcoming revision of the *United States Pharmacopeia* (U. S. P. XVI) will include infrared spectrophotometric identification tests for a considerable number of substances, both in bulk and dosage forms. In the past, identification of many pharmacopeial organic medicinals depended on color reactions produced with various test solutions; the utility of such tests was strengthened by specification of certain physical constants, and sometimes by quantitative evaluation procedures. It has long been obvious that for the large majority, if not all, substances, no single identification test may be considered as being specific for a substance. While ultraviolet spectrophotometric absorption characteristics are rather more specific, closely related substances all too often possess identical, or nearly identical, characteristics, in consequence nullifying the utility of this means of testing, both qualitatively and quantitatively. Increasingly, it has become apparent that identification tests applied to compounds are but tests for certain groups, such as carbonyl, hydroxyl, ketol, etc. Even such supposedly rigid criteria as melting point, refractive index, optical activity, and characteristics of derivatives may leave considerable doubt as to the identity of the compound being investigated. The infrared absorption spectrum of a substance, on the other hand, will *generally* provide conclusive proof as to its identity, and simultaneously will usually provide some indication of its purity. Even closely related compounds, indistinguishable or almost so by other tests, may be positively identified from their infrared spectrograms.

In developing infrared spectrophotometric identification tests for pharmacopeial use, consideration was given to three methods for establishing the identity of a substance, as follows: (1) to publish the infrared spectra of substances of U. S. P. grade for which this means of identification is to be utilized; (2) to specify the wave lengths at which absorption bands are observed for specific compounds, and to

indicate by the use of the terms *strong, moderate, weak,* and *shoulder* the intensity and character of the bands; (3) to specify that the spectrum of the substance to be tested shall conform with the spectrum, similarly determined, of a U. S. P. reference standard grade of the same substance. The third method was selected as the type to be used in U. S. P., this having the very important advantage of providing for the analyst the infrared spectrogram of the standard substance under the same experimental conditions employed in observing the substance being tested, thereby compensating for instrumental and procedural variations of testing which might otherwise vitiate the test.

The U. S. P. XVI official substances cortisone, hydrocortisone, hydrocortisone acetate, prednisolone, prednisolone acetate, and prednisone may be readily identified and differentiated by means of infrared spectrophotometry. These substances were found to give the most satisfactory spectra by the potassium bromide disc technique. The following specification, proposed as an identification test for prednisone in U. S. P. XVI, illustrates one type of infrared spectrophotometric procedure that will be used in many U. S. P. monographs: "The infrared absorption spectrum of a potassium bromide dispersion of Prednisone exhibits absorption maxima only at the same wave lengths as that of a similar preparation of U. S. P. Prednisone Reference Standard." It is noted that details of the method of conducting the test are not specified; these are left to the judgment and preference of the operator, the only requirement being that the spectrum of both the substance under test and that of the corresponding reference standard be determined similarly. While the wave length range of the spectrum is not specified in this test, the general directions for infrared spectrophotometric measurements in the appendix of the U. S. P. will specify a range of 2 to 14 microns for potassium bromide dispersions.

Figures 2 to 7 show the infrared absorption spectra of cortisone, hydrocortisone, prednisolone, prednisolone acetate, prednisone, and prednisone tablets. In the case of prednisone tablets, an extraction-purification procedure is applied in order to obtain crystals of sufficient purity for the infrared test. The proposed details of procedure for identification of the tablets are as follows: "Pulverize a number of Prednisone Tablets, equivalent to about 50 mg. of prednisone, and digest with 25 ml. of chloroform for 15 minutes. Filter the mixture, evaporate the filtrate on a steam bath to a volume of 2 to 3 ml., and then evaporate to dryness with the aid only of a current of air. Treat

the residue with two 10-ml. portions of hot solvent hexane, decanting the supernatant liquid each time and discarding it. Digest the residue with 25 ml. of dehydrated alcohol, warming slightly, for 15 minutes. Filter the hot solution, and evaporate the filtrate to a volume of 2 to 3 ml. Add solvent hexane until the mixture just becomes turbid, chill it to effect crystallization, collect the crystals, and dry them at 60° for 1 hour: the crystals respond to the Identification tests under Prednisone."

Similar tests, performed with potassium bromide dispersions of the test substance, make it possible to distinguish between the steroids desoxycorticosterone acetate and desoxycorticosterone trimethylacetate, and to differentiate testosterone cyclopentylpropionate, testosterone enanthate, and testosterone propionate; the tests will be used for identification of the respective substances in the new U. S. P. Other official substances to be identified by the same technique include: methotrexate, ouabain, progesterone, pyrimethamine, triethylene melamine, and tubocurarine chloride.

A variant of this procedure has been developed for the official antibiotics novobiocin calcium and novobiocin sodium and their respective dosage forms. The proposed procedure is as follows: "Dissolve about 100 mg. of Novobiocin Calcium in 30 ml. of water, transfer the solution to a separator, add 0.5 ml. of diluted hydrochloric acid, and extract the liberated novobiocin acid with 25 ml. of ether. Shake the ether extract with about 1 Gm. of anhydrous sodium sulfate, filter, evaporate the filtrate to dryness, and dry at 105° for 30 minutes: the infrared absorption spectrum of a potassium bromide dispersion of a portion of the residue exhibits absorption maxima only at the same wave lengths as that of a similar preparation of U. S. P. Novobiocin Reference Standard."

The U. S. P. antihistamines, both in bulk and in dosage forms, will be identified by a different infrared procedure, one in which the base component is extracted into carbon disulfide, this solution being used for the determination of the spectrum. The details of this procedure, developed by Federal Food and Drug Administration investigators, are proposed to be as follows: "Dissolve 50 mg. of the specified salt of the organic nitrogenous base, if in bulk, in 25 ml. of water, or shake a quantity of powdered tablets or the contents of capsules equivalent to 50 mg. of the salt with 25 ml. of 0.01 *N* hydrochloric acid for 10 minutes. Transfer the liquid to a separator, if

necessary filtering it through paper and washing the filter and residue with several portions of water. In a second separator dissolve 50 mg. of the corresponding U. S. P. Reference Standard in 25 ml. of water. Treat the two solutions identically, as follows: Add 2 ml. of sodium hydroxide T. S. and 4 ml. of carbon disulfide, and shake for 2 minutes. Centrifuge if necessary to clarify the lower phase, and filter it through a dry filter paper, collecting the filtrate in a small flask provided with a glass stopper. Determine the absorption spectrum of the filtrate in a 1-mm. cell in a suitable infrared spectrophotometer between 7 and 15 microns, using carbon disulfide in a matched cell as the blank. The spectrum of the solution prepared from the sample shows all of the significant absorption bands present in the spectrum of the solution prepared from the Reference Standard. If the spectrum of the sample preparation possesses obscuring absorption bands not present in that of the standard preparation, the sample may be further purified and the test repeated."

This identification test works satisfactorily with chlorcyclizine hydrochloride, chlorpheniramine maleate, diphenhydramine hydrochloride, phenindamine tartrate, promethazine hydrochloride, pyrilamine maleate, tripelennamine citrate and hydrochloride, and also the dosage forms of these antihistamines. Prochlorperazine ethanedisulfonate and prochlorperazine maleate and their dosage forms, newly admitted tranquilizer and anti-emetic drugs, are identified by the same type of test.

Some use will be made of liquid petrolatum mull dispersions, notably in identifying, and differentiating between, calciferol (vitamin D_2) and activated 7-dehydrocholesterol (vitamin D_3). The proposed wording of the official identification test is as follows: "The infrared absorption spectrum of a liquid petrolatum mull of Calciferol, in the range of 2 to 12 microns, exhibits the same absorption maxima and at the same wave lengths as that of a similar preparation of U. S. P. Calciferol Reference Standard." The reason for using liquid petrolatum as the dispersion medium, rather than one in potassium bromide, is because of the anomalous behavior that may be observed when these sterols are dispersed in the solid.

There is no doubt that utilization of infrared spectrophometric procedures for identifying many of the substances official in U. S. P. XVI has strengthened, probably as no other procedure could, the reliability of the identification test wherever this method is used, and

it is to be expected that other official substances will eventually be thus identified. While no quantitative infrared spectrophotometric procedure is presently under consideration for adoption in U. S. P. XVI, many official substances may be assayed by measuring the intensity of infrared absorption at a suitable wave length, and it appears reasonably certain that such assays are destined to appear in the Pharmacopeia in the near future.

Acknowledgments

The authors gratefully acknowledge the contribution by the Philadelphia College of Pharmacy and Science of personnel and laboratory facilities, including use of its Beckman IR-4 Recording Infrared Spectrophotometer, for this study undertaken on behalf of the U. S. P. Committee on Revision, and initiated by Arthur Osol as Chairman of U. S. P. Subcommittee No. 6. Grateful acknowledgment is recorded also for a research grant, made by the United States Pharmacopeial Convention, to one of the authors (Alfonso R. Gennaro).

THE EFFECTS OF FREEZE DRYING ON CERTAIN PHARMACEUTICAL PREPARATIONS [1, 2]

By Mahesh S. Mehta [3] and W. Lewis Nobles [4]

THIS study was undertaken in an effort to determine the effects of lyophilization on the physical and chemical properties of certain types of pharmaceutical preparations such as lotions, magmas, suspensions, elixirs, extracts, and gels. The lyophilized materials were reconstituted and their solubility, specific gravity, pH, viscosity, microscopical properties, and assay were compared with the similar properties of the original material.

Freeze-drying or lyophilization is now widely used as a commercial operation; twenty-five years ago, it was little more than a laboratory curiosity. This process consists of the rapid freezing of an aqueous solution of a material and subsequent removal of water by sublimation of the ice under high vacuum conditions.

In the early part of the twentieth century, this process was developed in the areas of drying sera, complement bacteria, and other biologicals (1, 2). The plasma program of World War II was a major factor in the development of this technique, and during World War II it was used extensively on a commercial scale for the processing of human convalescent serum, blood plasma, penicillin, and streptomycin (3-5). In recent years, water-soluble vitamins, hormones, enzymes, and other active medicinal agents have been lyophilized (6). This technique has also been utilized for the drying and preservation of plant materials (7-11) and for the processing of pharmaceutical gums and suspending agents (12).

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[1] Recipient of the Lunsford Richardson Pharmacy Award.

[2] Abstracted from a thesis submitted to the Graduate School of the University of Mississippi by Mehesh S. Mehta in partial fulfillment of the requirements for the degree of Master of Science.

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Experimental

(1) *Preparation of the material for lyophilization*

All preparations were made according to U. S. P. (13) and N. F. (14) methods.

(2) *Lyophilization of the material*

The lyophilizer was prepared for freeze-drying as previously described (15, 16). The condenser, freezing trap, and the freezing pans were all first cooled with acetone and dry ice. Twenty-five grams of the sample was weighed in each flask and the flasks were held vertically by clamps, placing them in the freezing pans perpendicularly (two flasks in each freezing pan). After standing for thirty minutes, they were then joined to the chamber of the lyophilizer. The lyophilizer was attached to a vacuum-pump and evacuated continuously for approximately thirty-six hours. At the end of the drying period, the pump was stopped. The flasks were taken from the apparatus, cleaned from the outside, weighed and then placed in the desiccator for overnight drying. After desiccation, the flasks were weighed and the percentage of solids obtained was calculated. The dry solids were scraped from the flasks and stored in well-closed bottles. This dry powder was used for subsequent tests.

(3) *Reconstitution*

One-half the weight of dry solids obtained was mixed with enough water or water-alcohol mixture to make 50 Gm. Lotions, magmas, and suspensions were reconstituted by triturating in a mortar; elixirs, extracts, and gels were reconstituted by stirring with a glass rod in a beaker. The rate of reconstitution was noted.

(4) *Suspension Test*

Fifty ml. of reconstituted sample was placed in a 100 ml. cylindrical graduate and compared with a 50 ml. aliquot of the original sample. Readings were taken at thirty-minute intervals and the time necessary for complete settling was noted.

(5) *Solubility Test*

Five grams of the lyophilized dry solids was weighed and put into a small beaker. Distilled water was added from a pipette and the mixture was stirred continuously. The volume of the solvent needed for complete solution was noted.

(6) *pH*

The pH of the reconstituted sample was determined immediately after reconstitution and, simultaneously, the pH of the original sample was determined. The pH of the same samples was taken after one-week and four-week intervals; see Table IV.

(7) *Specific Gravity*

The specific gravity was determined by the use of a Westphal balance. The instrument was tested with distilled water at 30°. The standard weight required for adjusting the instrument for water to a specific gravity of 1.0000 at room temperature was considered as one (sp. gr. of 1), and that weight was used for subsequent readings. With this adjustment of the instrument, readings of the original and the lyophilized samples were taken and their specific gravities were compared.

(8) *Viscosity*

The viscosity was determined by means of a Synchro-lectric viscometer. Spindles and speed were selected which gave the minimum difference in readings. After selecting the appropriate spindle and speed of rotation, an average of the best three of four or five readings was calculated.

(9) *Microscopical Examinations*

A few drops of the original and the reconstituted samples were examined under low power (100 magnification) to observe the microscopical characteristics of the samples.

(10) *Assays*

Assays were done according to U. S. P. and N. F. methods. Assays of the freshly reconstituted sample and of the original product were done at the same time. The assay of cascara sagrada fluid extract was carried out according to the method of Gibson (17). The percentage potency of the lyophilized sample was calculated in terms of the original sample as the standard (100%).

Discussion of Results

Table I summarizes the products lyophilized, their physical characteristics after lyophilization and the results of reconstitution and solubility tests. These data reveal that most of the preparations regenerated immediately into the original form with the exception of calamine lotion, benzyl benzoate lotion, and aluminum hydroxide gel. From Table I, it is also apparent that benzyl benzoate lotion and the elixir of phenobarbital could not be lyophilized to yield a solid form. In lyophilized preparations, most of the volatile constituents and flavors were lost, but a small amount of volatile material remained.

Relative viscosity determinations at 30° were taken on the original sample and the reconstituted sample immediately after regeneration. As can be seen from Table II, the viscosity of calamine lotion and aluminum hydroxide gel was considerably decreased due to lyophilization, while the viscosity of bentonite magma remained almost unaffected.

From Table II, it may be noted that the specific gravity of the lyophilized material was decreased in all samples. The specific gravity was considerably decreased in calamine lotion, elixir of phentobarbital, magnesia magma, and aluminum hydroxide gel. The specific gravity of the lyophilized aluminum hydroxide gel approached its normal value on homogenization.

From Table III, it may be seen that the suspensions prepared from the lyophilized samples of calamine lotion, benzyl benzoate lotion, magnesia magma, and aluminum hydroxide gel were not as satisfactory as the preparations which had been prepared according to the official formulas. In the aluminum hydroxide gel, the desirable properties of the original were regained upon homogenization. The lyophilized samples of bentonite magma, kaolin pectin mixture, and Sul-spansion ® regenerated to give suspensions similar to the original.

Table I
Time Percentage of Solids, Physical Characteristics, and Reconstitution

Category	Preparations	Time of Lyophilization	% of Solids	Physical characteristics after lyophilization	Reconstitution
I Lotions	Calamine Lotion (U.S.P. XV)	36 hrs.	17%	Light pink soft powder	Immediately with water viscosity is not gained
	Benzyl Benzoate Lotion (U.S.P. XV)	28 hrs.(D)	80% (liquid)	White liquid	Does not reconstitute into emulsion form
II Magmas	Bentonite Magma (U.S.P. XV)	36 hrs.	5%	Grayish white puffy powder	Immediately with warm water
	Magnesia Magma (U.S.P. XV)	36 hrs.	7%	White amorphous powder	Immediately with water
III Suspensions	Kaolin-Pectin Mixture (N.F. X)	36 hrs.	19.5%	Grayish white puffy powder with some smell of volatile oil	Immediately with water
	Sul-Spansion (S.K.F.) (S)	36 hrs.	55.5%	Solid pink flakes with odor of original flavor	Reconstitutes in five minutes
IV Elixirs	Phenobarbitel Elixir (U.S.P. XV)	36 hrs.	55% (liquid)	Red, thick, syrupy liquid	Immediately with alcohol-water mixture
	Aminoacetic Elixir Acid (N.F. X)	36 hrs.	25.8%	White, spongy, sticky material with volatile odor	Immediately with alcohol-water mixture
V Extracts	Fluidextract of Cascara Sagrada (N.F. X) (L)	36 hrs.	28.5%	Dark brown crystalline material	Immediately with alcohol-water mixture
VI Gels	Aluminum Hydroxide Gel (U.S.P. XV) (R)	36 hrs.	5.2%	Light soft white powder	Does not reconstitute into gel form

D - Due to shortage of dry ice.
S - Product of Smith, Kline and French Laboratories, Philadelphia.
L - Eli Lilly & Co. product.
R - Prepared according to method of Remington's Practice of Pharmacy, 11th Edition, p. 529.

From the microscopical examination, results of which are recorded in Table III, it may be seen that the emulsion of the benzyl benzoate lotion is broken permanently. In Sul-spansion ®, there is practically no change except some broken pellets which may have been disrupted in the process of reconstitution. In the lyophilized sample of aluminum hydroxide gel, particle size was tremendously increased.

Table II

Specific Gravity and Viscosity

Preparations	Specific Gravity / 30°		Viscosity cps / 30°	
	Original	Lyophilized	Original	Lyophilized
1. Calamine Lotion	1.155	1.084	950	37.5
2. Benzyl Benzoate Lotion	1.024	1.017		
3. Bentonite Magma	1.04	1.025	362	350
4. Magnesia Magma	1.0165	0.975		
5. Kaolin-Pectin Mixture	1.106	1.023		
6. Sul-Spansion	1.1005	1.045		
7. Phenobarbital Elixir	.865	.682		
8. Aminoacetic Acid Elixir	1.100	1.0935		
9. Cascara Sagrada Fluidextract	1.074	1.025		
10. Aluminum Hydroxide Gel	1.3695	1.01[b]		
11. Aluminum Hydroxide Gel	1.3695	1.339[a]	1,680	340[a]

(a) Homogenized
(b) Not homogenized

Table III

Suspension, Solubility and Microscopical Examinations

	Preparations	Suspension or Solubility		Microscopical Examinations	
		Original	Lyophilized	Original	Lyophilized
1.	Calamine Lotion	Settles in 8 hours	Settles in 1 hour		
2.	Benzyl Benzoate Lotion	Settles in 7 hours	Settles in 30 min.	Uniform and oily globules regular in shape	Not uniform. Irregular oily globules
3.	Bentonite Magma	Does not settle	Does not settle		
4.	Magnesia Magma	Settles in 7 hours	Settles in 3 hours		
5.	Kaolin-Pectin Mixture	Does not settle	Does not settle		
6.	Sul-Spansion	Does not settle	Does not settle	Thousands of uniformly packed pellets	Nearly the same Some broken pellets
7.	Phenobarbital Elixir				
8.	Glycine Elixir		1 Gm. dissolves in 2 ml. of water		
9.	Cascara Sagrada Fluidextract		1 Gm. dissolves in 2 ml. of water		
10.	Aluminum Hydroxide Gel	Does not settle	Settles[b] in 45 min.	Uniform, particle size smaller	Less uniform and particle size larger[b]
11.	Aluminum Hydroxide Gel				Smooth, uniform and particle size slightly larger than that of original[a]

(a) Homogenized
(b) Not homogenized

It appears to be evident from the data in Table IV that the pH of most of the lyophilized materials was somewhat higher immediately after reconstitution than that of the original samples. This may be due in some cases to the fact that there is a loss of carbon dioxide during lyophilization with a subsequent elevation of the pH of the reconstituted sample (18). After a period of four weeks, the pH closely approximated that of the original in most of the preparations. The pH of calamine lotion prepared from the lyophilized material was lower than that of freshly prepared calamine lotion both immediately after reconstitution and after four weeks.

From the results of the assays reported in Table V, it is revealed that there is practically no difference in the potency of lyophilized aminoacetic acid elixir and phenobarbital elixirs while there is a decrease in the magnesium hydroxide content of the lyophilized material in magnesium magma. Considering some loss in lyophilization and experimental errors (from lyophilization through assay), this decrease in potency is negligible. There is recorded an unexplainable increase in the content of the active principle in the lyophilized samples of the aluminum hydroxide gel in both the homogenized and non-homogenized ones.

From the results of the assay of the cascara sagrada fluid extract, there is noted a difference of thirty-three per cent in the content of free anthraquinones, as compared with a decrease of eleven per cent in combined anthraquinones. During the assay processes of the lyophilized samples, it was observed that the lyophilized sample of aminoacetic acid elixir gave a sharp end point in the formation of the formaldehyde aminoacetic acid complex compared with the original. The combined anthraquinones of lyophilized cascara sagrada fluid extract sample gave a distinct bright and immediate zone in the chromatographic column with magnesium oxide [m] and celite [c] compared with the original.

The lyophilized material after three months' storage at room temperature remained the same in appearance and color in all preparations with the exception of aminoacetic acid elixir. The lyophilized powder in aminoacetic acid elixir was discolored and the reconstituted sample also changed color.

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[m] Adsorptive magnesia sea-sorb 43. Fisher Scientific Co.

[c] Celite (hyflo super cel). Johns Mansville Co.

Table IV

pH

Preparation	Original			Lyophilized		
	Fresh	After 1 week	After 4 weeks	Fresh	After 1 week	After 4 weeks
1. Calamine Lotion	11.5	11.5	11.1	10.7	10.1	8.2
2. Benzyl Benzoate Lotion[a]						
3. Bentonite Magma	9.7	9.2	7.9	9.7	9.0	6.9
4. Magnesia Magma	9.7	9.9	9.2	9.8	10	9.2
5. Kaolin-Pectin Mixture	3.7	3.7	3.4	3.9	3.8	3.4
6. Sul-Spansion	5.2	5.2	5.0	5.2	5.2	5.0
7. Phenobarbital Elixir	5.9	5.5	4.6	6.1	6.0	4.8
8. Aminoacetic Acid Elixir	5	4.8	4.3	5.2	5.1	4.3
9. Cascara Sagrada Fluidextract	4.4	4.4	4.2	4.5	4.5	4.2
10. Aluminum Hydroxide Gel	9.3	9.0	8.3	9.7[c]	9.3	8.3
11. Aluminum Hydroxide Gel				9.7[b]	9.5	9.1

(a) pH was not taken.
(b) Homogenized.
(c) Not homogenized.

Summary

From the results of the lyophilization of different pharmaceutical preparations studied in this investigation, the following tentative conclusions may be drawn:

(1) The lyophilized preparations are immediately regenerated to yield preparations approximating the original form with the exception of calamine lotion, benzyl benzoate lotion, and aluminum hydroxide gel upon treatment with a suitable solvent.

(2) The viscosity and specific gravity of lyophilized preparations are decreased compared with the original.

(3) The suspending property of the media is decreased in calamine lotion, benzyl benzoate lotion, magnesium magma, and aluminum hydroxide gel, while it is apparently not affected in bentonite magma, kaolin pectin mixture, and Sul-spansion®. It is regained upon homogenization in aluminum hydroxide gel.

(4) The pH of the lyophilized material is increased immediately after reconstitution but, after a four weeks' period, it closely approximated that of the original in most of the preparations, while the pH is greatly decreased in calamine lotion immediately after reconstitution and after four weeks.

(5) There is apparently some difference in potency due to lyophilization in some cases; this was observed in the case of aluminum hydroxide gel in which the content of the active principle of the lyophilized sample was apparently increased. This observation may well have been due to an experimental error in assay, although this result was obtained also on repetition of the work. A decrease in the anthraquinone glycoside content was noted in the cascara sagrada assay.

(6) Emulsion type lotions and liniments are not suitable for lyophilization.

(7) Suspensions, magmas, and water soluble extracts yield excellent products upon lyophilization.

(8) Elixirs such as phenobarbital elixir which contain a smaller percentage of solids and a higher percentage of glycerin, syrup, and

Table V

I. Assay

	Preparation	Original (a)	Lyophilized (b)	% of Potency of Lyophilized (c)
1.	Magnesia Magma	5.73% of $Mg(OH)_2$	4.67% of $Mg(OH)_2$	87%
2.	Phenobarbital Elixir	428 mg. of phenobarbital in 100 ml. of elixir	408 mg. of phenobarbital in 100 ml. of elixir	95%
3.	Aminoacetic Acid Elixir	12.92% of $C_2H_5NO_2$	12.56% of $C_2H_5NO_2$	97.2%
4.	Aluminum Hydroxide Gel	1.31% of Al_2O_3	1.67% of Al_2O_3 [e]	127.6%
			1.58% of Al_2O_3 [d]	120.6%

(a) Average of two readings.
(b) Average of two readings.
(c) Based on considering potency of original 100%.
(d) Homogenized.
(e) Not homogenized.

II. Assay - Results of Cascara Sagrada Fluidextract
Calculated weight in Gm. / 5 ml. of Anthraquinones

Sample	Free Anthraquinones	Combined Anthraquinones	Total	% of Potency
Original	.0003 gms.	.0082 gms.	.0085 gms.	100%
Lyophilized	.0002 gms.	.0073 gms.	.0075 gms.	88.2%

alcohol are not suitable for lyophilization, while elixirs like aminoacetic acid elixir which contain a high percentage of solids and a smaller percentage of syrup, alcohol, and glycerin may be subjected to lyophilization.

(9) Calamine lotion and aluminum hydroxide gel may be lyophilized if the change in physical and chemical properties have not altered the therapeutic efficiency.

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ALKALOIDAL IODIDES—THEIR PRESCRIPTION INCOMPATIBILITIES

(A Preliminary Study)

By Leroy Honkomp * and J. Leon Lichtin **

Background

THE precipitate produced when either alkali metal iodides or hydriodic acid is present in aqueous solution along with alkaloidal salts has been mentioned as a general incompatibility in pharmaceutical texts (1-8).

Among the factors said to increase the likelihood of precipitation is a greater concentration of the alkaloid (1, 4), a greater concentration of the iodide (3, 5, 6) or a greater concentration of both (7, 8). The alkalinity of solutions of sodium or potassium iodide has been blamed as the offender in this type of precipitate. It has been claimed that these salts are purposely manufactured to produce alkaline solutions in order to retard the appearance of free iodine. In the reacting of such solutions with alkaloidal salts, the precipitate would therefore be the free insoluble alkaloid or perhaps a mixture of it with the insoluble alkaloidal iodide (2-5).

It has been suggested that the formation of the precipitate can be retarded by the following conditions: (a) the presence of alcohol (2)—with a concentration of 15% to 20% being specified (7, 8); (b) the dissolving of the iodide and the alkaloidal salt each separately in a little water followed by diluting each with a fraction of the vehicle and, lastly, followed by mixing of the two solutions (4, 6).

Application has been made of the alkaloid-iodide precipitate in the development of microcrystalline qualitative tests for several of the alkaloids including codeine (9), apomorphine (10), benzylmorphine (11), brucine (12), and quinidine (13).

Lastly, it has been noted by the authors that students occasionally report that prescriptions containing a codeine salt and an iodide precipitated upon being compounded or subsequently upon standing. Prescriptions of this type also have been noted in journal columns devoted to "prescription problems".

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Because of the mention of this type of incompatibility in the case of codeine and of the importance of both the antitussive alkaloids and iodides in the treatment of cough, two antitussive substances—codeine and dihydrocodeinone—were chosen as the alkaloids in this investigation. An examination has been made of the conditions under which these alkaloids precipitate as insoluble iodides. Variables considered were the concentration and manner of mixing the ingredients, the presence of alcohol, the presence of two proprietary cough mixtures, and the presence of alkali.

Materials

(1) The "Water" used throughout these experiments was distilled water.

(2) Codeine Phosphate (U. S. P. Merck).

(3) Potassium Iodide A. R. Mallinckrodt.

(4) Hydriodic Acid C. P.—47% w/w, Coleman and Bell.

(5) Potassium Bicarbonate A. R. Mallinckrodt.

(6) Potassium Carbonate N. F. Merck.

(7) Dihydrocodeinone Bitartrate N. F. Merck.

(8) Cheracol ® (Upjohn, supplied by courtesy of Mr. E. V. Falter).

(9) Mercodol ® (Merrell, supplied by courtesy of Dr. F. Joseph Murray).

Note (a)—Observation Periods: The mixtures were observed for about two hours after the initial mixing, and then daily for three to four days. Following this, observations were made one week afterwards, and then at about two-week intervals for about one to two months.

Note (b)—Nature of the Codeine Iodide Precipitates: Unless otherwise noted, these were always crystalline, which can be described as X-crossed clusters of needles. These closely resembled the published description of codeine iodide (9).

Note (c)—Time for Precipitate to Form: The crystalline precipitates usually formed on the day the experiment was performed or within forty-eight hours—in all cases, within four days. The more concentrated solutions were generally more prompt in producing precipitates. Some amorphous precipitates, in insignificant amounts, formed only after many days.

Experimental Data and Results

(1) *Codeine Phosphate and Potassium Iodide Solutions*

The codeine salt and the iodide were each dissolved in 30 ml. of water. The potassium iodide solution was added to the alkaloidal salt solution. The final concentrations of the alkaloidal salt per 30 ml. of mixture were 16, 32, 65, 130, and 260 mg., and the concentrations of the iodide per 30 ml. of mixture were 0.9, 1.8, 3.6, and 7.2 Gm.

Results: Precipitation occurred where the concentration in the final mixture of the codeine salt was 260 mg./30 ml. and the iodide was 0.9 and 1.8 Gm./30 ml.

(2) *Codeine Phosphate and Hydriodic Acid Solutions*

Thirty ml. portions of aqueous solutions of hydriodic acid were added to 30 ml. portions of codeine phosphate so that the final concentrations of the alkaloidal salt per 30 ml. of mixture were 16, 32, 65, 130, and 260 mg., and those of hydrogen iodide were 0.23, 0.45, 0.67, and 0.9 Gm. (Note: The 0.23 Gm./30 ml. of mixture corresponds to the U. S. P. Hydriodic Acid Syrup.)

Results: The highest concentration (only) of codeine phosphate (260 mg./30 ml.) produced a precipitate when the final HI concentration was either 0.67 or 0.9 Gm. per 30 ml.

(3) *Codeine Phosphate Solution and Hydriodic Acid in Syrup*

This group of experiments was performed in a manner similar to those of the previous group with the following exceptions: (1) a still higher concentration of hydriodic acid was added to the series (1.35 Gm. HI per 30 ml. of mixture; (2) the required amount of hydriodic acid was diluted with Syrup U. S. P. to 30 ml.

Results: Similar to those obtained using aqueous solutions of hydriodic acid. The highest concentration of codeine phosphate (260 mg./30 ml. of mixture) produced precipitates with concentrations of HI of 0.67 Gm. or more per 30 ml.

(4) *Codeine Phosphate and Potassium Iodide in the Presence of Cheracol®*

The various amounts of codeine phosphate and potassium iodide were dissolved in 5 ml. and 30 ml. of water, respectively. The iodide

was added to the codeine salt, and immediately sufficient Cheracol was added to make a final volume of 60 ml. The concentrations per 30 ml. of the final mixture were as follows: codeine salt (in addition to that contained in Cheracol)—32, 64, 130, and 260 mg.; potassium iodide—0.9, 1.35, and 1.8 Gm.

Results: Only in the one mixture containing the highest concentration of each ingredient did the characteristic crystalline precipitate occur.

(5) *Codeine Phosphate, Hydriodic Acid in Syrup, and Cheracol®*

This group of experiments was performed in a manner similar to those of the previous section with the following exceptions: Hydriodic Acid was used as the iodide; the amount required was diluted with Syrup to 30 ml. The concentrations per 30 ml. of the final mixture were as follows: codeine salt (in addition to that contained in Cheracol)—32, 65, 130, and 260 mg.; hydrogen iodide—0.23, 0.9, and 1.35 Gm.

Results: No crystalline precipitates formed. An amorphous precipitate, requiring eight to eleven days to form, appeared in most of the mixtures containing the two higher concentrations of HI. This precipitate differed from the usual codeine iodide as follows: It was dark brown (instead of yellow); the amount formed (especially when dried) was much smaller; the material was not soluble in acetone or ethyl alcohol (as contrasted to codeine iodide which was). It was concluded that no alkaloidal iodide was produced in this group of experiments. *Note:* Control Experiments were performed using several of the higher concentrations of codeine phosphate along with Cheracol and, in the place of the iodides, potassium chloride and hydrochloric acid. The KCl controls produced no precipitate. All the samples containing the HCl produced a slowly forming amorphous precipitate which is believed (as in the case of HI) not to involve the alkaloid. The higher concentrations of HCl produced more of this precipitate.

(6) *Dihydrocodeinone Bitartrate (DB) and Potassium Iodide Solutions*

Each of the reactants was dissolved in 30 ml. of water. The iodide was added to the alkaloidal salt. The concentrations per 30

ml. of the final mixtures were as follows: DB—30, 60, 120, and 240 mg.; KI—0.9, 1.35, 1.8, 3.6, and 7.2 Gm.

Results: In the mixtures containing 240 mg. DB/30 ml., a concentration of 1.35 Gm. KI/30 ml. of mixture produced a crystalline precipitate. When the concentration of DB was reduced to 120 mg. per 30 ml. of mixture, it required a concentration of 3.6 Gm. of KI to produce a similarly appearing precipitate. This had the form of colorless small crystals of approximately prismatic shape.

Further experiments are being conducted to determine whether this precipitate could be utilized as the basis of a microchemical test for dihydrocodeinone salts.

(7) *Dihydrocodeinone Bitartrate (DB) and Hydriodic Acid Solution*

These experiments were done in a manner analogous to those described in Section 6. The HI was present in water to make 30 ml. of solution. The concentration per 30 ml. of the final mixtures were as follows: DB—30, 60, 120, and 240 mg.; HI—0.9 and 1.35 Gm.

Result: No precipitate occurred in any of the mixtures.

(8) *Dihydrocodeinone Bitartrate (DB) and Hydriodic Acid in U. S. P. Syrup*

These experiments were done in the manner described in the previous group except that Syrup was the vehicle for the HI. The concentrations of the ingredients in 30 ml. of the final mixtures were as follows: DB—7.5, 15, 30, and 60 mg.; HI—0.23, 0.67, 0.9, and 1.35 Gm.

Result: No precipitates occurred in any of these.

(9) *Dihydrocodeinone Bitartrate (DB), Hydriodic Acid (in Syrup) in the Presence of Mercodol*

The DB was dissolved in 5 cc. of water. To this was added 25 ml. of Mercodol, followed by mixing. The HI, contained in sufficient Syrup to make 30 ml., was then added, and mixed well. The concentrations used of the DB and the HI per 30 ml. of final mixture were the same as in the previous section. *Note:* The DB in Mercodol actually contributed an *additional* 4.2 mg. per 30 ml. of mixture.

Result: No precipitates formed.

(10) *Terpin Hydrate Elixir Along With Potassium Iodide or Hydriodic Acid (in U. S. P. Syrup)*

Codeine alkaloid was dissolved in 30 ml. of the elixir. The KI was dissolved in 30 ml. of water. (Hydriodic acid was diluted with Syrup to make 30 ml. of solution.) The iodide was added to the elixir and mixed well. The concentrations of the ingredients in 30 ml. of the final mixtures were as follows: Codeine (as *added* codeine)—0, 30, 60, and 130 mg.; KI or HI—0.23, 0.45, 0.9, and 1.35 Gm.

Results: The samples containing KI underwent no marked change. All the samples containing HI slowly turned dark, and a dark-colored substance gradually rose to the top. (This may be due to a reaction of HI with certain volatile constituents.) No precipitate at all formed in any of the mixtures in this group.

(11) *Concentration of the Ingredients and the Time Required for Precipitation*

1.8 Gm. of KI and 520 mg. of codeine phosphate were dissolved separately in equal (but varying) volumes of water. The solution of the iodide was then added to that of the alkaloidal salt. The time at which the formation of the codeine iodide precipitate occurred was noted.

Results:

ml. of Solution of Each Ingredient	*Time of Precipitation*
2.5	Instantly
5	About 15 seconds
10	About 45 seconds
15	2 minutes
20	13 minutes
30	3 days
40	No precipitate after 3 weeks
50	No precipitate after 3 weeks
60	No precipitate after 3 weeks

(12) *Influence of Alcohol Upon Time Required for Precipitation*

Control: 1.8 Gm. of KI and 520 mg. of codeine phosphate were each dissolved separately in 20 ml. of water. About ten minutes later, precipitation occurred.

Solutions of 20 ml., containing the same concentration of KI and of codeine phosphate, were mixed, immediately followed by the addition of quantities of ethyl alcohol which varied from 5 to 30 ml.

Results:

ml. of Added Ethanol	*Final % of Ethanol in Mixture*	*Result*
5	11	Precipitate in 3 days
10	20	No precipitation
20	33	No precipitation
30	43	No precipitation

The above experiment was repeated using the same amounts of alkaloidal salt and KI *with the exception* that they were each dissolved in *30 ml.* of water. To these mixtures were then added the following amounts of alcohol: 5, 10, 20 and 30 ml. The percentages of alcohol thus ranged from 8% to 33%.

Results: No precipitation occurred in any of the mixtures. A control containing no alcohol produced a precipitate in three days.

(13) *Effect of Alkalis*

Thirty ml. portions of 10% Potassium Bicarbonate and of 10% Potassium Carbonate were added to 30 ml. portions of varying concentrations of codeine phosphate so that the concentration per 30 ml. of the final mixtures were as follows: Codeine Phosphate—65, 130, and 260 mg. The alkalis were thus present in 5% concentration. The pH of the bicarbonate-containing mixtures was about 8.2-8.4. The pH of the carbonate-containing ones was about 9.0 to 9.5.

Results: No precipitate appeared in three weeks.

(14) *The pH of Potassium Iodide Solutions*

Using a glass electrode and indicator solutions, it was determined that the U. S. P. grades of solutions of potassium iodide in concentrations varying from 1 to 10% produced a pH in the range of 8.1 to 9.2. The pH of the solutions of the analytical reagent grade of potas-

sium iodide were in the range of 7.7 to 8.3. These figures were confirmed as being essentially correct by correspondence with the control department of the Mallinckrodt Chemical Works (courtesy of Mr. C. S. Kettler).

Conclusions

1. The mixing of equal volumes of aqueous solutions of codeine phosphate with those of HI or KI was not followed by the formation of a crystalline precipitate characteristic of codeine iodide, *provided that* in each 30 ml. of the final mixture there was 130 mg. or less of the codeine salt mixed with 7.2 Gm. or less of KI, or 1.35 Gm. or less of HI. If, however, the concentration of the codeine salt was 260 mg./30 ml., then 0.6 to 0.9 Gm. of HI or KI would produce a precipitate.

2. The mixing of equal volumes of aqueous solutions of dihydrocodeinone bitartrate (DB) with those of HI or KI was not followed by the formation of a crystalline precipitate *provided that* in each 30 ml. of the final mixture there was 60 mg. or less of DB mixed with 7.2 Gm. or less of KI or 1.35 Gm. or less of HI. If the alkaloidal sale were increased to 120 mg./30 ml., then 3.6 Gm. of KI would produce a precipitate. A level of 240 mg./30 ml. of DB precipitated with only 1.35 Gm. of KI/30 ml.

3. In the presence of the two cough proprietary mixtures studied, the tendency for the formation of the codeine iodide was slightly lessened.

4. If the quantities are sufficient for the formation of a precipitate, these two antitussive iodides will form within four days in aqueous preparations. The more usual therapeutic concentrations of the alkaloids and the iodides do not form precipitates.

5. The concentration of the ingredients markedly affects the time at which precipitation occurs. This confirms the suggestion of texts that, in compounding with these substances, they should be present in as dilute a state as possible when mixing them.

6. It has been confirmed that alcohol in 10% to 20% concentration markedly inhibits this type of precipitation.

7. The formation of the precipitate between the two antitussive alkaloids and KI or HI appears to be independent of the slight alkalinity of potassium iodide solutions. Two points to support this can be offered:

(a) There appears to be no evidence that HI has less tendency than KI to precipitate these alkaloids.

(b) These alkaloids appear to be much more readily precipitated by solutions of potassium iodide (analytical reagent with solution pH near 8) than by solutions of potassium bicarbonate (pH 8 to 9) or of potassium carbonate (9 to 10).

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BOOK REVIEW

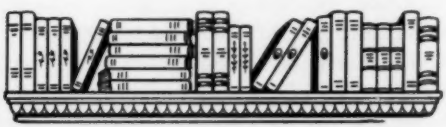
The Chemistry of Organic Medicinal Products, Fourth Edition. G. L. Jenkins, W. H. Hartung, K. E. Hamlin, and J. B. Data. x + 569 pp. John Wiley and Sons, Inc., New York 16, N. Y., 1957. Price: $10.75.

This new edition of the now familiar "Jenkins and Hartung" is a complete revision of the third edition, accompanied by the acquisition of two new co-authors. The original format is still retained; that is, the compounds are discussed according to functional group rather than physiological activity. To some readers, this idea may not sit well, but it is the most logical approach since compounds of one type may have many physiological actions and, therefore, the necessary duplication would tend to make the book unwieldy.

Each of the sixteen chapters begins with a brief discussion of the topic under consideration, followed by short paragraphs on individual compounds. Both official and proprietary names are included, along with some pharmacological data.

Little synthetic material has been included, but ample literature references should satisfy those particularly interested in this phase of medicinal chemistry. This book makes for ideal reading, especially the chapter on physiochemical properties. Many new ideas correlating structure with physiological ideas are presented to the reader. A good index rounds off this fine edition of an extremely useful book.

A. R. GENNARO



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